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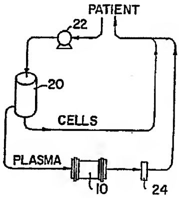

(57) Abstract

A method for treating cancer by conducting extracorporeal perfusion of blood plasma from a cancer patient through a device, such as a column (10), which contains absorbent matrix material including phosphorylcholine (PC) or PC derivatives so as to remove C-reactive protein and anti-PC antibodies and thereby improve the cellular immune responses of the patient against the cancer. The method may be employed as a sole treatment or in combination with another modality for treating cancer such as treatment with IL-2 or other cytokines.

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PROCESS FOR REMOVING C-REACTIVE PROTEIN AND ANTI-PHOSPHORYLCHOLINE ANTIBODIES FROM BIOLOGICAL FLUIDS

BACKGROUND OF THE INVENTION

Field of the Invention

The present invention relates to a method for removing C-reactive protein and antiphosphorylcholine antibodies from biological fluids to improve the cellular immune responses thereof, and specifically to a method for removing C-reactive protein and antiphosphorylcholine antibodies from the circulation of patients with cancer by conducting extracorporeal perfusion of a patient's blood plasma through a phosphorylcholine-matrix adsorption device so as to improve the patient's cellular immune responses against the cancer. The method of the present invention may be employed alone or in combination with other cancer treating modalities, such as interleukin 2 or other cytokines.

Description of the Related Art

Various methods have been disclosed for the extracorporeal treatment of blood in order to remove various substances, such as immunoglobulins, from the blood. For example, the use of protein A as an immunoadsorbent in extracorporeal immunotherapy affinity columns is disclosed by Messerschmidt et al, J. Clin. Oncol., Vol. 6, No. 2, (Feb. 1988), pp. 203-212. Heat and formalin-treated Staphylococcus aureus Cowan I packed in a column has been employed for the removal of IgG from the blood as disclosed in Holohan et al (1982) Cancer Res. 42:3663-3668. Another system in which protein A is entrapped within a charcoal matrix and utilized to treat the plasma is disclosed by Terman et al (1981) N. Engl. J. Med. 305:1195-1200. Other blood treatment systems for the removal of anti-A and anti-B antibodies in which the immunoadsorption system utilizes synthetic human blood group antigens covalently linked to a silica matrix are disclosed in Bensinger et al (1981) N. Engl. J. Med 304:160-162; and Bensinger et al (1982) J. Clin. Apheresis 1:2.5. The use of a protein A-silica column

for extracorporeal immunoadsorption is also briefly reported by Bensinger et al (1982) N. Engl. J. Med. 306:935. The use of carbodiimide coupling to Sepharose is discussed in "Affinity Chromatography-Principles and Methods," published by Pharmacia Fine Chemicals, Uppsala, Sweden.

Yet a further method for the extracorporeal treatment of blood is disclosed by U.S. Patent 4,681,870 to Balint, Jr. et al in which an immunoadsorbent material for removing IgG and IgG-complexes from biolgoical fluids is prepared by covalently binding protein A to a solid-phase silica matrix, and is employed in a column for therapeutic treatment of various cancers and autoimmune disorders where IgG-complexes are implicated as suppressing factors in inhibiting a normal immune response.

Although the above-noted methods provide for a variety of extracorporeal blood treatment alternatives, it is still very desirable to provide additional novel treatment methods in order to further enhance therapeutic effects associated with the treatment of cancer and other diseases.

The clinical use of adoptively transferred natural effector cells activated by recombinant human interleukin-2 (IL-2) has revealed a fundamental paradox: although natural effector cells, such as lymphokine-activated killer (LAK) cells, lyse nearly any tumor cell in vitro, extremely small lysis rates are exhibited by the adoptively transferred effector cells in vivo. Some of the discrepancy may be explained by the apparent failure of the adoptively transferred effector cells to infiltrate tumor sites. However, even when the effector cells are placed into direct contact with tumor cells in patients with tumors restricted to the abdominal cavity, only a minority of the patients respond to the treatment. Steis et al, "Intraperitoneal Lymphokine-Activated Killer Cell and Interleukin 2 Therapy for Peritoneal Carcinomatosis: Toxicity, Efficacy and Laboratory

Results," Proc. Am. Soc. Clin. Oncol. (1987), 6:250.

The failure of effector cells to kill tumor cells when placed in direct contact with the tumor cells in vivo could reflect the presence of host effector which interferes with tumor cells recognition or killing by the adoptively transferred effector cells and the host defense cells present in the body. A number of experimental studies have supported the idea that tumor cells themselves produce or elicit the production by the host of "tumor protecting agents" or "blocking" or "suppressor" factors. Hellstrom et al, "Blocking (Suppressor) Factors, Immune Complexes, and Extracorporeal Immunoadsorption in Tumor Immunity," Contemp. Top. Immunobiol. (1985), 15:213; Israel et al, "Plasmapheresis in Patients with Disseminated Cancer: Clinical Results and Correlation with Changes in Serum Proteins: The Concept of Non-specific Blocking Factors," Cancer (1977), 40:3146; Bansal et al, "Blocking and Unblocking Serum Factors in Neoplasia," Curr. Top. Microbiol. Immunol. (1976), 75:45. Transient clinical responses have been observed in patients undergoing plasma exchange to remove these factors. Israel et al, supra. The precise nature of the blocking factors has not been determined but immune complexes, monomeric IgG molecules, and a number of other blocking factors have been proposed. Rao et al, "Specific In Vivo Inhibition of Macrophage Receptors for Cytophilic Antibody by Soluble Immune Complexes," Cancer Res. (1979), 39:174; Pislarasu et al, "Modulation of Natural Killer Cell Activity by Serum from Cancer Patients: Preliminary Results of a Study of Patients with Colorectal Adenocarcinoma or Other Types of Cancer," Cancer Res. (1988), 48:2596.

It is therefore further desirable to identify other blocking factors which interfere with host cell lysis of tumor cells and to develop a method for removing the blocking factors in order to enhance tumor cell lysis.

SUMMARY OF THE INVENTION

Therefore, it is an object of the present invention to provide a method for removing C-reactive protein and antiphosphorylcholine from biological fluids so as to improve cellular immune responses against cancer.

It is the further object of the present invention to remove soluble C-reactive protein and antiphosphorylcholine antibodies from the blood of cancer patients by extracorporeal perfusion of their blood plasma through a phosphorylcholine matrix adsorption device so as to improve cellular immune responses against the cancer.

It is yet another object of the present invention to provide a system for pumping blood from a patient to a cell separator which separates the blood into cell and plasma components, which comprises the steps of passing the plasma component through a device which contains an adsorbent matrix material including phsophorylcholine so as to remove C-reactive protein and antiphosphorylcholine antibodies thereby producing treated plasma, recombining the treated plasma and cell components to produce treated blood, and returning the treated blood to the patient.

The foregoing objects and others are accomplished in accordance with the present invention, generally speaking, by providing a method for the extracorporeal treatment of biological fluids, such as human blood and blood plasma, which comprises removing C-reactive protein and antiphosphorylcholine antibodies from said biological fluids so as to improve cellular immune responses against cancer. The present invention further provides a method and system for employing the same, which comprises pumping blood from a patient through a cell separate which separates the cells into blood cells and plasma, passing the blood plasma through a device, such as a column, which contains adsorbent

matrix material that includes phosphorylcholine for removing C-reactive protein and antiphosphorylcholine antibodies, and recombining the plasma and cells before returning the same to the patient.

Further scope of the applicability of the present invention will become apparent from the detailed description given hereinafter. However, it should be understood that the detailed description, accompanying figures, and specific examples, while indicating preferred embodiments of the invention, are given by way of illustration only, since various changes and modifications within the spirit and scope of the invention will become apparent to those skilled in the art from this detailed description.

BRIEF DESCRIPTION OF THE DRAWINGS

The present invention will become more fully understood from the accompanying Drawings which are provided for illustration only, and thus are not limitative of the present invention, and wherein:

Figure 1 is a schematic suggestion for C-reactive protein involvement in the macrophage recognition and killing of tumor cells;

Figure 2 is a diagrammatic representation of a system for the extracorporeal treatment of blood which may be used to carry out the method of the present invention;

Figure 3 is a graph indicating the influence of C-reactive protein when it is combined with IL 2-stimulated human serum or ascites fluid with regard to rosette formation;

Figure 4 is a graph indicating the influence of the antibody to C-reactive protein when it is added to IL 2-stimulated human serum with regard to rosette formation; and

Figures 5(A) and 5(B) are graphs indicating the percent lysis of tumor cells in various mixtures including IL 2-stimulated human serum, IL 2-stimulated ascites

fluid, and C-reactive protein before and after the mixtures are passed over a phosphorylcholine column.

DESCRIPTION OF THE PREFERRED EMBODIMENTS

The inventor of the present invention has observed that C-reactive protein is expressed on the membrane of human macrophages and that the presence of interleukin 2 (IL 2) in human plasma induces a significant increase in C-reactive protein (CRP) expression on macrophage membrane, and further that the presence of either soluble CRP, or antiphosphorlcholine (anti-PC) antibodies in a killing mixture of macrophages and target tumor cells almost completely blocks the killing capacity of the effector cells, even after activation with IL2. Performing such experiments in ascitic fluid from a patient with peritoneal carcinomatosis totally blocks the cytotoxic capacity of the effector cells. However, removal of soluble CRP and anti-PC antibodies from the ascitic fluid by means of a phosphorylcholine affinity column totally restores the cytotoxic capacity of the effector cells. These observations suggest that CRP is being used by macrophages to recognize and to bind to tumor cells selectively followed by their destruction. The existence of significant amounts of circulating CRP as well as anti-PC antibodies in cancer patients interferes with the cell mediated cytotoxicity of the immune system. One suggestion for CRP involvement in macrophage killing of tumor cells is schematically represented in Figure 1. Thus, removal of CRP and anti-PC antibodies from the circulation of a patient should improve therapeutic efficacies. This removal may be conducted by employing a method for the extracorporeal treatment of biological fluids in which the fluids are passed through a CRP-adsorbent device, such as a column, having an appropriate adsorbent matrix contained therein in accordance with the present invention.

In order to perform the method of the present invention, a CRP-adsorbent material therein is provided

for the extracorporeal treatment of a biological fluid, such as blood plasma, in order to remove CRP and anti-PC antibodies therefrom. The treatment may be conducted by continuously removing a patient's blook separating the blood cells therefrom, treating the separated plasma in the CRP-adsorbent column or device so as to remove the CRP and anti-PC, and mixing and returning the treated plasma and blood cells directly to the patient. Alternatively, after the blood has been removed from the blood cells separated, the blood cells may be directly reinfused into the patient. The separated plasma may be collected, treated in the CRP-adsorbent column, again collected, and then returned to the patient as early as possible.

The CRP-adsorbent material contained in the column which is used in the method of the present invention comprises phosphorylcholine (PC) or PC derivatives bonded to a matrix so as to maximize the activity of the PC or PC derivative and the binding capacity of the column or device, while minimizing leakage of the PC and the PC derivatives, as well as other substances, from the column during use. Generally, an effective amount of PC or PC derivatives is used in preparing a column. For example, a few milligrams of PC per gram of matrix material may be used. One example of a non-limiting range is from about 0.6 to 1.6 mg of PC or PC derivative per gram of column matrix material. In one preferred embodiment, the PC or PC derivatives are cross linked to amino groups of a formatted silicon matrix so as to be capable of removing CRP and anti-PC antibodies to improve cellular immune responses against cancer.

The PC and PC derivatives useful in the method of the present invention include all PC derivatives that sufficiently bind CRP and anti-PC antibodies. Examples of PC derivatives include PC esters, such as p-nitrophenyl-6-(O-phosphorylcholine)hydroxy hexanoate.

The matrix contained in the extracorporeal

device or column used in the method of the present invention may be formed from any material suitable for carrying the PC and PC derivatives, such as silicon, Agarose, Sepharose, acryloid beads, other suitable polymeric substances and matrixes, and solid-phase silica. The solid-phase silica matrix may comprise virtually any form of particulate silica including amorphous silicas, such as colloidal silica, silica gels, precipitated silicas, and fumed or pyrogenic silicas; microcrystalline silicas such as diatomites; and crystalline silicas such as quartz. The silica should have a particle size in the range from about 45 to 120 mesh, usually in the range from about 45 to 60 mesh. Other materials useful for forming a matrix as disclosed in U.S. Patent 4,681,870 may also be used in the device or column employed in the method of the present invention. U.S. Patent 4,681,870 is herein incorporated by reference.

The PC and/or PC derivatives are bound to the device or column matrix in a suitable manner so as to retain the ability of the PC and PC derivatives to remove CRP and anti-PC from the biologiccal fluid passed over the column matrix. For example, the PC or PC derivatives may be cross linked to amino groups of a formatted silicon matrix. Other methods for binding the PC or PC derivatives to the matrix material may be used such as those applicable methods disclosed by U.S. Patent 4,681,870.

The method of the present invention may be carried out by employing an appropriate extracorporeal device or column containing the CRP-adsorbent matrix material as described above. A removable cartridge may be contained within the column for containing the adsorbent matrix material therein. An example of such a cartridge is described in U.S. Patent 4,681,870. As shown in Figure 2, a system for conducting the extracorporeal treatment method of the present invention includes a column or device 10 which is connected to a

cell separator 20. The column or device 10 may be sterilized, for example with a gas sterilant such as ethylene oxide, and either used immediately or sealed and stored for later use. Prior to use, the column or device 10 may be washed with normal saline followed by a wash with normal saline containing any other suitable preparatory ingredients. However, no calcium ion chelating agents should be introduced.

The column or device 10 is then connected to the cell separator 20 to receive separated plasma therefrom. The cell separator 20 may be continuous flow cell separator, such as an IBM Model 2997, available from IBM, or may comprise a semi-permeable membrane which allows passage of the plasma and blood proteins, but prevents passage of the cellular elements of the blood. In the case of a semi-permeable membrane which allows passage of the plasma and blood proteins, but prevents passage of the cellular elements of the blood. In the case of a semi-permeable membrane, a blood pump 22 is used to pass the blood through the membrane. Suitable blood pumps include a tube and a peristalic pump wherein the blood is isolated from the pumping machinery to prevent contamination. The blood passes through the cell separator 20 at a rate which may be in the range of from about 10 to 20 ml/min. typically until a total desired volume of blood has been passed. The blood cells are mixed with the plasma passing through the treatment column or device 10, and the recombined blood returned to the patient. A microfilter 24 may be provided at the outlet of the treatment column or device 10 to prevent passage of macroscopic particles which might be lost from the column or device 10.

EXPERIMENTAL

Preparation of Materials

Malignant ascites (tumor cells confirmed by microscopic examination) were withdrawn from the peritoneal cavities of nine patients after obtaining their

informed consent. Five patients had primary carcinoma of the colon, two had ovarian cancer, one had pancreatic cancer, and one had mesothelioma.

The ascitic cells were then isolated. Freshly obtained ascite specimens were centrifuged in 250 ml sterile polypropylene containers at 1200 rpm for 10 minutes at 4°C. The cell-free ascitic fluid was harvested and kept at 4°C until used. The cell pellet was washed three times in phosphate-buffered saline (PBS) supplemented with 0.4% sodium citrate. After the third wash, the cells were resuspended in a small volume of cold RPMI 1640 and layered over Ficoll. After centrifugation for 30 minutes at 1800 rpm at room temperature, the interphase cell layer was collected, washed twice in RPMI 1640, and resuspended at 10^8 cells/ ml in RPMI 1640 with 2% pooled human heat-inactivated AB serum, 50 μg/ml gentamicin, and 2mM glutamine. The cells were then kept at 4°C until use.

Tumor cytotoxicity was quantified based on the following procedures. Ficoll-enriched, washed ascite cells were incubated under a variety of experimental conditions at 37°C in humidified air with 5% CO_2 with gentle agitation at a concentration of 3-4 x 10^6 cells/ml. Controlled incubations were performed at 4°C. Samples of 100 microliters (304 x 10^5 cells) were withdrawn from the culture at various time points and slides were made by spinning the sample in a cytocentrifuge, fixation in methanol, and staining with Giemsa. A total of 10 low-power (25x) fields were examined (2 slides, 5 fields each). A total number of tumor cells in these fields were enumerated and the fraction of cells forming rosettes (at least 4 cells directly attached to the tumor cell) was determined. A semi-quantitive assessment of the degree of rosette formation was formulated based on the percentage of tumor cells forming rosettes and the number of host effector cells surrounding each tumor cell. The criteria were as follows:

4+ = all tumor cells form rosettes, all rosettes comprised of at least 8 cells;

3+ = > 80% of tumor cells form rosettes, > 80% rosettes comprised of at least 8 cells;

2+ = > 80% of tumor cells form rosettes, 20-80% of rosettes comprised of at least 8 cells, the remainder having 4-7 cells;

1+ = 20-80% of tumor cells form rosettes, < 20% of rosettes comprised of at least 8 cells, 80% or more having 4-7 cells;

+/- = < 20% of tumor cells form rosettes, all rosettes comprise 4-7 cells; and

0 = no rosettes or few partial rosettes.

Cytotoxicity was determined by harvesting cells cultured under various experimental conditions for 24 hours and making Giesma-stained cytocentrifuge preparations, as noted above for assessment of rosette formation. Surviving tumor cells were identified morphologically and counted in 10 low-power fields (2 slides, 5 fields each). Duplicate samples were evaluated for each culture condition. Parallel cultures were incubated at 4°C for the same time period and were considered negative controls. Cytotoxicity was calculated as follows:

$$\text{Cytotoxicity} = \left(1 - \frac{(\text{avg. number of surviving tumor cells at } 37^\circ\text{C})}{(\text{avg. number of surviving tumor cells at } 4^\circ\text{C})}\right) \times 100$$

C-reactive protein was isolated and purified from malignant ascites by a modification of the procedure described by Volanakis et al, "C-reactive Protein: Purification by Affinity Chromatography and Physiochemical Characterization," J. Immunol. Meth. (1978), 23:285; and Oliveira et al, "comparative Studies on the Binding Properties of Human and Rabbit C-reactive Protein," J. Immunol. Meth. (1980), 124:1396. The C-reactive protein so obtained formed a single band on polyacrylamide gel electrophoresis, binded to antibodies to C-reactive protein, and was stored at -70°C at 1.5 mg/ml in PBS.

C-reactive protein was biotinylated according to the procedure of Bayer et al, "The Avidin-Biotin Complex in Affinity Cytochemistry," Meth. Enzymol. (1979), 62:308, and was stored at 4°C in a 1 mg/ml solution. Unlabelled (lot 25962) and fluorescein isothiocyanated (FITC-labelled) (lot 12217) $F(ab')_2$ fragments of sheep anti-human C-reactive protein antibodies were purchased from Cappel (Melvern, PA), dialyzed against PBS and sterilized by filtration before use. Murine monoclonal IgM antibodies 17/207 (obtained from Dr. J. Kenny, PRI, NCI-FCRF) and HPCM2 (obtained from Dr. P. Gearhart, Johns Hopkins University) specific for phosphorylcholine were stored at 4°C in 1 mg/ml sterile solution. The anti-phosphorylcholine antibodies were biotinylated by the method of Bayer et al, supra. Recombinant human IL 2 was obtained from the Cetus Corp. (Emeryville, CA).

Cytospin slides that had been fixed in cold acetone (minus 20°C) for 45 minutes were coated with FITC-labelled $F(ab')_2$ fragments of antibodies to C-reactive protein, biotinylated antiphosphorylcholine antibodies, or biotinylated C-reactive proteins for 90 minutes at room temperature. Biotinylated antibodies were developed with a second incubation of 60 minutes with FITC-labelled avidin. The slides were washed in PBS buffer containing 0.2% albumin, 100 mg/L Ca^{++}, 100 mg/L mg^{++}, and 0.01% sodium azide, and the slides were counterstained with 0.5% Evans blue dye. The slides were then examined with a fluorescence microscope.

The levels of C-reactive protein were measured by radial immuno diffusion. A phosphorylcholine affinity column was prepared using 50 mg of P-nitrophenyl-6-(O-phosphocholine)hydroxy hexanoate, prepared by "molecular probes" in accordance with the procedure of T.F. Spande, J. Organic Chem. (1980) 45: 3081, which was dissolved in 0.2 ml of dry acetonitrile and the resulting solution then added to 60 ml of a 1:1 suspension of -aminohexyl agarose (Sigma) in borate buffer saline (BBS), at pH 8.4. This

mixture was stirred gently overnight at 4°C and washed with 1 liter of BBS. The washed PC-matrix was packed in a glass column 2 cm in diameter and about 15 cm in height. The column was then used to remove CRP and anti-PC antibodies from human malignant ascites.

Results:

The malignant ascites were examined under the microscope after Giemsa staining, and abundant malignant cells, lymphocytes, macrophages, neutrophils, and a few mesothelial cells were observed. There were very few tumor cells interacting with the host defense cells present in the ascitic fluid, regardless of the tumor cell type or the number and type of host cells present. Cells from 8 of the 9 patients were incubated for one hour at 37°C either in autologous cell-free ascitic fluid or an RPMI 1640 with 2% human AB serum. Spontaneous rosette formation in the ascites averaged a score of 0.49 +/- 0.06. However in serum-containing media, the value was 1.86 +/- 0.16. Thus, the rosette formation in the serum-containing medium was significantly higher than that in the ascites. Spontaneous rosette formation in the ascites was not different from the levels seen in the serum-containing medium incubated at 4°C. Rosette formation was significantly increased by incubating the ascitic cells for one hour at 37°C in serum-containing media supplemented with 500 U/ml recombinant human IL 2. The effect of added IL 2 on rosette formation in the ascitic fluid was considerably less dramatic, though still significantly increased over spontaneous rosette formation in the ascitic fluid. Thus, the ascitic fluid significantly inhibited both the spontaneous and the IL 2-induced rosette formation among tumor and host defense cells.

Ascitic cells were then incubated for one hour at 37°C in autologous ascites and the autologous ascites depleted of C-reactive protein by passage over a column of immobilized phosphorylcholine. As shown in Figure 3, IL 2-stimulated rosette formation was 3 times higher in

ascites from which C-reactive protein had been removed and was similar to the IL 2-stimulated rosette score observed in the serum. In order to confirm that the blocking factor was C-reactive protein and not another undefined phosphorylchlorine binding protein simultaneously removed by the phosphorylcholine column, purified C-reactive protein was added to the ascitic cell cultures after these cultures had been depleted of C-reactive protein. As shown in Figure 3, purified C-reactive protein inhibits IL 2-stimulated rosette formation in either ascites or in a serum-containing medium although not completely to the level of the pre-column ascites. Thus, the C-reactive protein is apparently capable of blocking the interaction between host defense cells and malignant cells in the ascitic fluid. If C-reactive protein is the effector's targeting device, antibodies to C-reactive protein should also block rosette formation. As shown in Figure 4, the IL 2-stimulated formation of tumor rosettes was significantly inhibited by antibodies to C-reactive proteins. The antibodies completely blocked the stimulatory effects of the IL2, reducing IL 2-stimulated rosette formation to the level of spontaneous rosette formation in the serum. To confirm that macrophages express C-reactive protein, cytocentrifuge preparations of ascitic cells were stained with FITC-conjugated $F(ab')_2$ fragments of antibodies to C-reactive protein. The macrophages expressed abundant cell surface molecules recognized by the antibodies to C-reactive protein. Collectively, this data demonstrates that tumor rosette formation in malignant ascitic cells depends upon the interaction between cell surface C-reactive protein on macrophages and tumor cells expressing phosphorylcholine.

In order to access the functional impact of rosette formation on the tumor cells, ascitic cells were cultured under a variety of conditions for 24 hours and the number of surviving tumor cells was determined by counting the cells on a Giemsa-stained cytocentrifuge

preparation. Tumor cells death was considered to be at the baseline level (i.e., 0% killing) in cultures incubated at 4°C, i.e., conditions that interfere with rosette formation. The formula for calculating cytotoxicity as described above incorporates this assumption. As demonstrated in Figure 5(A) and 5(B), the lytic effect on tumor cells incubated for 24 hours precisely paralleled the propensity to form rosettes. Figures 5(A) and 5(B) are graphic representations of the results of photomicrographs at 25 x of Giemsa-stained ascitic cells cultured under a variety of conditions for eight different patients, each patient being represented by a different symbol. These symbols include a circle, an hourglass shape, a triangle directed upwards, a triangle directed downwards, a star, a diamond shape, a blank square, and a square having two crossed diagonal lines. Both killing and rosette formation occur in serum but not in ascites. Addition of IL 2 enhances both killing and rosette formation in serum but the effect is substantially less imperative in ascites. IL 2-stimulated rosette formation and killing reached a level comparable to that seen in serum when C-reactive protein was first removed from the ascites by passage over a phosphorylcholine column. Addition of either purified C-reactive protein or antibody to C-reactive protein inhibits both rosette formation and killing in either serum or ascites. A correlation coefficient for rosette formation and cytotoxicity was determined to be 0.85 ($p<0.05$). Thus, rosette formation is a reasonably accurate surrogate measure for tumor cytotoxicity. The above observations were confirmed by objective measurements of cytotoxicity in isotope released assays using human macrophages and tumor cell lines and it was directly shown that besides C-reactive protein, anti-PC antibodies also block killing, while removal of anti-PC antibodies restores tumor cell killing.

The invention being thus described, it will be obvious that the same may be varied in many ways. Such

variations are not to be regarded as a departure from the spirit and scope of the invention, and all such modifications as would be obvious to one skilled in the art are intended to be included within the scope of the following claims.

WHAT IS CLAIMED IS:

1. A method for the extracorporeal treatment of biological fluids which comprises:

removing C-reactive protein and antiphosphorylcholine antibodies from said biological fluids so as to improve cellular immune responses against cancer.

2. The method of claim 1, wherein said biological fluids comprise human blood and blood plasma.

3. The method of claim 2, wherein said C-reactive protein and antiphosphorylcholine antibodies are removed by passage of said human blood and blood plasma through a device which contains an adsorbent matrix that includes phosphorylcholine.

4. The method of claim 3, wherein said adsorbent matrix comprises phosphorylcholine disposed in a matrix substrate material selected from the group consisting of formatted silicon, Sepharose, acryloid beads, Agarose and solid phase silicia.

5. A method for extracorporeal treatment of human blood so as to improve cellular immune response which comprises:

pumping blood from a patient to a cell separator;

separating the blood into cell and plasma components;

passing said plasma component through a device which contains adsorbent matrix material including phosphorylcholine for removing C-reactive protein and antiphosphorylcholine antibodies from said plasma component so as to produce treated plasma;

recombining said treated plasma with said cell component so as to produce treated blood; and

returning said treated blood to said patient.

6. The method of claim 5, wherein said adsorbent matrix comprises phosphorylcholine disposed on a matrix substrate material selected from the group

consisting of formatted silicon, Sepharose, acryloid beads, Agarose and solid phase silica.

7. A system for the extracorporeal treatment of blood so as to improve cellular immune response which comprises:

a column or device for receiving blood plasma therethrough which contains an adsorbent matrix material that includes phosphorylcholine for removing C-reactive protein and antiphosphorylcholine antibodies from said blood plasma.

8. The sytem of claim 7, further comprising

a blood pump for pumping blood from a patient;

a cell separator connected to said pump for receiving said blood and for separating said blood into cell and plasma components;

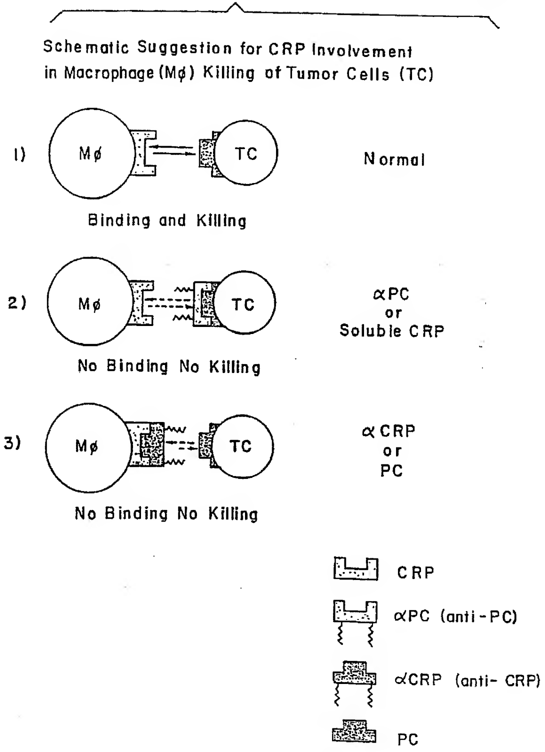
said column being connected to said cell separator for receiving said plasma component therefrom and for treating said plasma by passage therethrough so as to produce treated plasma; and

a microfilter for preventing passage of macroscopic particles from said column or device before said treated plasma is recombined with said cell component to produce treated blood which is returned to said patient.

9. The system of claim 8, wherein said adsorbent matrix comprises phosphorylcholine disposed on a matrix substrate material selected from the group consisting of formatted silicon, Sepharose, acryloid beads, and solid phase silica.

10. The method of claim 1, which further comprises treating said biological fluids with at least another modality for treating cancer.

FIG. 1


Schematic Suggestion for CRP Involvement
in Macrophage (Mø) Killing of Tumor Cells (TC)
1)
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αCRP (anti- CRP)
PC

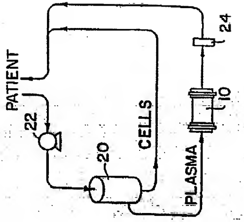
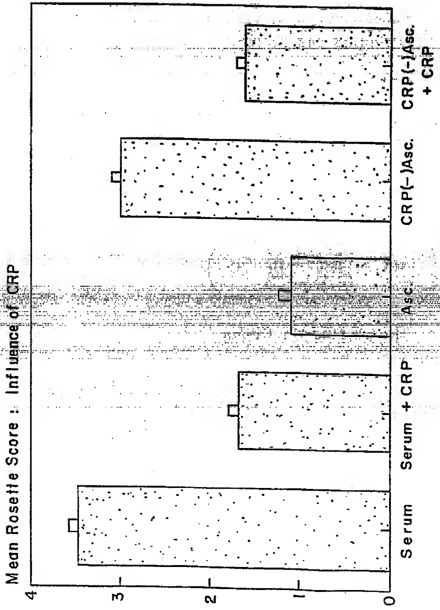


FIG. 2



(IL2-stimulated)

FIG. 3

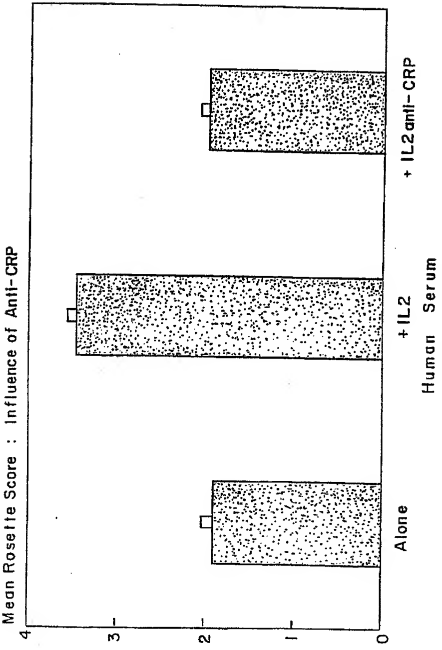

Mean Rosette Score : Influence of Anti-CRP
4
3
2
1
0
Alone
+IL2
+IL2 anti-CRP
Human Serum

FIG.4

FIG. 5(A)

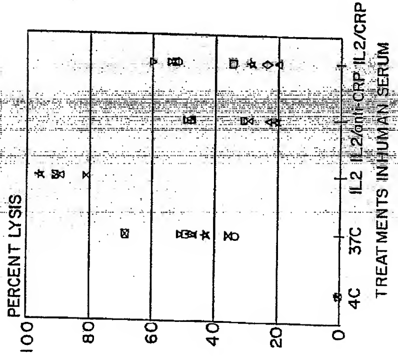
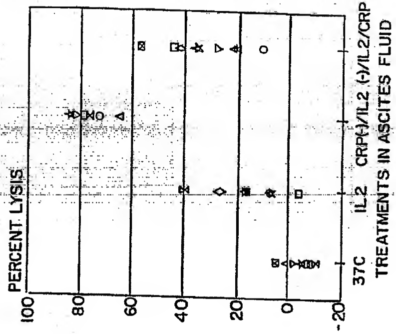

PERCENT LYSIS
100
80
60
40
20
0
4C
37C
IL2
IL2/anti-CRP
IL2/CRP
TREATMENTS IN HUMAN SERUM

FIG. 5(B)


PERCENT LYSIS
100
80
60
40
20
0
-20
37C
IL2
CRP(-)/IL2
(-)/IL2/CRP
TREATMENTS IN ASCITES FLUID

INTERNATIONAL SEARCH REPORT

International Application No. PCT/US90/01873

I. CLASSIFICATION OF SUBJECT MATTER (if several classification symbols apply, indicate all) [6]

According to International Patent Classification (IPC) or to both National Classification and IPC

IPC(5): B01D 15/08
U.S. Cl: 210/635,656,679,691,198.2,502.1; 530/380,387,830;604/5

II. FIELDS SEARCHED

Minimum Documentation Searched [7]

Classification System	Classification Symbols
U.S.	210/635,656,679,691,198.2,263,502.1 530/380,387,830 604/4,5,6

Documentation Searched other than Minimum Documentation to the Extent that such Documents are Included in the Fields Searched [8]

III. DOCUMENTS CONSIDERED TO BE RELEVANT [9]

Category *	Citation of Document, [11] with indication, where appropriate, of the relevant passages [12]	Relevant to Claim No. [13]
Y	US, A, 4,681,870 (BALINT) 21 July 1987 see column 1, lines 8-29	1-10
Y	US, A, 4,203,893 (PERY) 20 May 1980 see column 6, lines 14-28 and column 12, lines 7-12	1-10
Y	US, A, 4,775,483 (MOOKERJEA) 04 October 1988 see column 2, lines 9-12	1-10
Y	US, A, 4,384,954 (NAKASHIMA) 24 May 1983 see column 2, lines 65-66	1-10
Y	US, A, 4,472,303 (TANIHARA) 18 September 1984 see column 4, line 50	1-10

* Special categories of cited documents: [10]

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier document but published on or after the international filing date

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"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

"&" document member of the same patent family

IV. CERTIFICATION

Date of the Actual Completion of the International Search	Date of Mailing of this International Search Report
04 May 1990	14 JUN 1990
International Searching Authority	**Signature of Authorized Officer**
ISA/US	Ernest G. Therkorn

Form PCT/ISA/210 (second sheet) (Rev.11-87)